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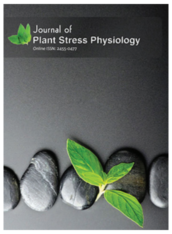

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Efficacy of jerangau merah (*Boesenbergia stenophylla* R.M. Smith) crude root extracts for suppressing *Collectotrichum gloeosporiodes* Penz. associated disease of chili (*Capsicum annum* L.)

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ABSTRACT

Environmental pollution issues have prompted the exploration of biological control as a promising alternative for managing diseases in chili plants. However, the use of plant extracts and microbial inoculants to promote growth and control diseases in chili, particularly in Malaysia, especially Sarawak, is limited. The primary objective of this study was to assess the potential of *Trichoderma* and *Boesenbergia stenophylla* R.M Smith in suppressing *Colletotrichum gloeosporioides* Penz., a pathogen associated with chili (*Capsicum annum* L.) diseases. The efficacy of *B. stenophylla* in inhibiting *C. gloeosporioides* showed a maximum PIRG (Percent Inhibition Relative to Control Growth) value of 86.26% on day 8. The formulation of *B. stenophylla* (jerangau merah) demonstrated potential in suppressing chili anthracnose disease both *in vitro* and under field conditions. Two *Trichoderma spp*. isolated from the soil of rehabilitated forest floors were evaluated for their *in vitro* antagonism against *C. gloeosporioides*. *T. harzianum* gradually inhibited the growth of *C. gloeosporioides* starting from day 2, completely overtaking it by day 8 with a PIRG of 87.40%. *T. harzianum* inoculants proved effective in controlling the pathogen in vitro and enhancing the growth of chili seedlings, in addition to inhibiting *C. gloeosporioides*. Meanwhile, *T. longibrachiatum* also gradually inhibited the growth of *C. gloeosporioides*, achieving a PIRG of 56.02% by day 8. The presence of *Trichoderma* in the rhizosphere and on the roots generally improved the root growth of chili seedlings compared to controls inoculated with sterile distilled water (SDW) and those treated with *B. stenophylla* extracts. Chili seedlings responded better to *T. harzianum* inoculants than to *T. longibrachiatum* inoculants and *B. stenophylla* extracts. By week 8, seedlings inoculated with *T. harzianum* showed the highest root growth with 26.87 cm in root length and 9.48 g in root fresh mass. Disease assessment studies indicated that *T. harzianum* exhibited the greatest potential as a biocontrol agent (BCA), reducing disease incidence and severity by 53% and 51%, respectively. Similarly, application of *B. stenophylla* powder slowed down infection progression and improved chili plant growth, with disease incidence and severity values of 72% and 60%, respectively. Overall, the study demonstrated the efficacy of *B. stenophylla* in protecting and enhancing the growth of chili plants, potentially replacing harmful chemicals. Both *B. stenophylla* and *T. harzianum* inoculants showed effectiveness against *C. gloeosporioides*, suggesting their potential development as biocontrol agents. Assessment of plant-microbe interactions indicated that *T. harzianum* mediated induced resistance by producing inducible compounds such as peroxidase (PO). Single inoculation with *T. harzianum* was most effective, followed by a mixture of *T. harzianum* + *B. stenophylla*, delaying symptom onset and reducing disease incidence and severity. In conclusion, the findings suggest that *Trichoderma* inoculants and *B. stenophylla* extract powder are effective against *C. gloeosporioides* while promoting plant growth. Further research into formulation, application frequency, and techniques is essential to maximize their potential as BCAs against *Colletotrichum* diseases in chili plants.

Keywords: Biological control, Chili (*Capsicum annum* L.), *Collectotrichum gloeosporioides*, Crude root extract, Jerangau merah (*Boesenbergia stenophylla* R.M. Smith)

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INTRODUCTION

Colletotrichum gloeosporioides is considered one of the most significant plant pathogens globally causing the economically important disease anthracnose. The diseases are mainly problematic on mature chili fruits, causing severe losses due to pre and post-harvest fruit decay (Boseland & Votaven, 2003). In Malaysia, anthracnose fruit rot stands out as a notable concern, particularly affecting chili production. An accession of chili has exhibited robust resistance to two key pathogens, namely C. gloeosporioides and C. capsici. The infection by C. gloeosporioides typically initiates in leaves and then spreads to flowers, leading to blossom blight that can destroy inflorescences and result in significant yield reduction.

In Malaysia, research focused on chili anthracnose and its association with *Colletotrichum* has primarily centered on *C. truncatum* as the identified causal pathogen (Mahmodi *et al*., 2014). However, earlier investigations have indicated that multiple *Colletotrichum* species can infect chili fruits. Hence, there is a possibility that more than one *Colletotrichum* species contributes to chili anthracnose in Malaysia (Harp *et al*., 2008). Furthermore, recent taxonomic research on *Colletotrichum* species has identified numerous new species within *Colletotrichum* species complexes.

The extensive use of fungicides has led to the accumulation of harmful chemicals that pose potential risks to both humans and the environment. Additionally, this practice has contributed to the development of resistance to pathogens. It's worth noting that concerns about fungicide resistance in various fungal species, including *Colletotrichum spp*. have arisen. These concerns have emphasized the need to seek more environmentally friendly and sustainable alternatives (Avila-Adame *et al*., 2003).

Therefore, exploring and utilizing biological control agents (BCAs) seem to be a highly promising approach. Biological control is receiving increasing attention as an alternative means of disease control, both pre- and postharvest, especially where disease resistance or chemical control is not available. Biocontrol involves the utilization of naturally occurring non-pathogenic microorganisms and the manipulation of plant extracts, which can reduce the activity of plant pathogens, thereby effectively suppressing diseases. Consequently, employing biocontrol agents to manage this pathogen can contribute to increased crop yields. Biological control, which involves introducing antagonistic microorganisms into the soil to combat soilborne plant pathogens, holds promise as a nonchemical approach to plant disease management.

In response to these concerns, recent efforts have been directed toward developing environmentally friendly and effective biocontrol agents, as well as plant-based products. Therefore, there has been a growing interest in exploring natural plant products as potential alternatives to fungicides, as they have the potential to be less toxic and safer for the environment (Yoon *et al*., 2001).

There are additional instances where plant extracts have demonstrated a proven inhibitory effect on the mycelium growth of phytopathogens. In biological activities, *Boesenbergia rotunda* exhibited antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antispasmodic, antitumor and insecticidal activities (Tewtrakul *et al*., 2003). *Boesenbergia stenophylla* plants belong to the Zingiberaceae family and are known for antimicrobial properties. Some studies show that the family containing phenols and phenolic compounds, cyanogenic glycosides, flavonoids and lignans, leuocoanthocyanins, catechol, tannins, galangal, naptho-quinone and coumarin (Kasarkar & Kulkarni, 2011). Another genus *Boesenbergia spp* was studies to control *collectrotrichum* of mango fruit and avoid fruit damage (Khewkhom *et al*., 2012). *Boesenbergia spp*. are use as Biocontrol agents to control fungal plant diseases as well as other fungicides alternatives. The general objective of this study was to evaluate the potential *B. stenophylla* crude root extracts in suppressing *C. gloeosporioides* associated disease.

MATERIALS AND METHODS

Dual Culture Test of *Boesenbergia stenophylla* Crude Root Extracts against *Collectrotrichum gloesporoides*

The crude roots of *B. stenophylla* were pulverized into powder form using a grinder and then packed into polyethylene bag and stored in desiccators at room temperature (28±2 °C). Ten grams of *B. stenophylla* powder were added to 90 mL of distilled water and shaken for one hour. Then, a 10 mL aliquot of this suspension was transferred aseptically to another 90 mL of distilled water. One mL of this diluted suspension was then inoculated onto Potato Dextrose Agar (PDA) plates. The solidified agar plates were subsequently incubated at 27 °C for 8 days. The control plates contained only *C. gloeosporoides*. Disks (5 mm in diameter) of *B. stenophylla* were inoculated on PDA medium on one side of the petri dish, with *C. gloeosporoides* inoculated on the opposite side. The radial mycelial growth of *C. gloeosporoides* was measured daily from day 1 to day 8 of the incubation period at 28±2 °C.

B. stenophylla Crude Root Extracts in Suppressing *Collectotrichum gloesporoides* and Inducing Biochemical Responses on Chili

Sterilized cocopeat was filled into sets of cultivation pots (10 kg pot^{-1}). Nylon meshes (<1.0 mm) were cut into squares and fitted to the bottom of each cultivation pot to prevent medium loss. The cocopeat was amended with 10^8 c.f.u. mL^{-1} of *C. gloesporoides* culture suspension and incubated for 2 weeks. *B. stenophylla* powder and applied to the polybags of cocopeat infested with *C. gloesporoides* at planting time. Hundred grams of *B. stenophylla* powder and 10^8 x c.f.u g^{-1} air dried preparation were applied as media treatment (worked into the top 5 cm of soil). Polybags were sown with 6 weeks old chili seedlings.

Disease development was monitored based on quantitative assessment measured as disease incidence (Vanderplank, 1984). Disease incidence (DI) refers to the number of seedlings visibility infected in relation to the total number of seedlings assessed. Estimation based on disease incidence is the quick set and easiest method, and suitable for wilt disease whereby one lesion unit seedlings^{-1} is considered fatal (Campbell & Maddeen, 1990). The formula used to calculate disease incidence is:

$$\%\ \text{Disease Incidence (DI)} = \frac{\text{number of diseased seedlings}}{\text{total number of seedlings assessed}} \times 100\%$$

Infected seedlings were also assessed for their disease severity. Disease severity (DS) refers to the volume of the cutting tissue that is infected relative to the total volume of the seedlings. It is expressed as the percentage of seedlings tissue with symptoms of disease at a particular recording time. Infected seedlings may produce varying degrees of severity, based on the extent of the intensity of foliar infection, intensity of foliar yellowing, defoliation, collar infection and wilted vines. Disease severity was rated based on the disease assessment whereby each scale denotes the extent of symptoms displayed due to *Collectotrichum* infection:

A=Leaves, anthracnose generally appears first as small, irregular yellow or brown spots. These spots darken as they age and may also expand, covering the leaves.

B= Fruits, it produces small, dark, sunken spots, which may spread. In moist weather, pinkish spore masses form in the center of these spots. Eventually, the fruits will rot.

C= trees, it can kill the tips of young twigs.

The anthracnose symptoms in the genotypes were assigned to scale values such as 0 = no infection, 1 = 1-2% of fruit area with necrotic lesion, 3 = >2-5% of fruit area with necrotic lesion, 5 = >5-15% of fruit area with necrotic lesion, 7 = >15-25% of fruit area with necrotic lesion and 9 = >25% of fruit area with necrotic lesion as described earlier (Montri *et al.*, 2009).

RESULTS AND DISCUSSIONS

Dual Culture Test of *B. stenophylla* Crude Root Extracts against *C. gloesporoides*

The antagonistic activity for *B. stenophylla* was established in the bioassay treatment. From the result the test revealed that *B. stenophylla* gradually inhibited the growth of *C. gloesporoides* from day 2 till day 8 (Table 1).

B. stenophylla exhibited 80% inhibition on day 6, which increased to 86.26% by day 8. Despite not reaching 100% inhibition, these results are promising and indicate that the active compounds or phytochemical contents in *B. stenophylla* can effectively inhibit the growth of *C. gloeosporoides*. Ahmad and Jantan (2003) identified methyl (E)-cinnamate in the essential oils of *B. stenophylla*, which has antimicrobial properties, including antibacterial effects against *Escherichia coli*. PIRG values are valuable in determining the efficacy of an isolate as a potential biocontrol agent (BCA); higher PIRG values (>50%) indicate stronger antagonistic activity. While in vitro studies may not always correlate perfectly with field observations, they are crucial for screening the antagonistic potential of microorganisms. This experiment underscores the potential of *B. stenophylla* as an effective biocontrol agent, warranting further investigation into their application and mechanisms in disease management.

Table 1: Percentage Inhibition of Radial Growth (PIRG) of *Boesenbergia stenophylla* crude root extracts against *Colletotrichum gloesporoides*

Day	% Inhibition of Radial Growth *B. Stenophylla*
1	0[a]
2	31.3[a]
3	45[b]
4	57.65[b]
5	76.03[a]
6	80.2[a]
7	84.36[a]
8	86.26[a]

Mean with same letters within the same row are not significantly different between treatments ($DNMRT_{0.05}$)

Efficacy of *Boesenbergia stenopyhlla* Crude Root Extracts in Suppressing *Collectorichum gloesporoides* and Inducing Biochemical Responses on Chili

The phenomenon of systemic acquired resistance is relatively a new strategy for controlling plant disease. The resistance was evident as a reduction in disease incidence compared with infected control. Treatment with *B. stenophylla* protected chili plants against anthracnose disease. The plants treated with SDW (Control), appeared unhealthy. The progress and extent of symptom development from week 1 until week 12 in the field was observed. This observation suggests the potential of *B. stenophylla* in chili plants, which delayed the onset of symptoms, as well as lessen disease severity. Analysis showed that different application of *B. stenophylla* had different effect and their subsequent effect in delaying the onset of disease progression. There were significant differences in DI% and DS% values recorded (Table 2).

Based on the results from the disease assessment study presented in Table 3, the development of disease symptoms was generally slower in treatments T1 compared to T2 and T3. The control group exhibited the highest disease incidence (DI%) and disease severity (DS%), both recorded at 100%. In contrast, treatments T2 and T3 showed lower values in DI%

Table 2: Different treatments on chili plants for disease assessment

Treatment	Description
T1	*B. stenophylla* 100%
T2	*B. stenophylla* 70%
T3	*B. stenophylla* 50%
T4	Control (SDW)

Table 3: Disease incidence (%) and Disease severity (DS%) recorded in chili plants of different treatment at week 12 (Values are mean of eight replicates)

Treatment	DI (%)	DS (%)
T1	53[d]	51[b]
T2	89[b]	79[a]
T3	72[c]	60[b]
Control	100[a]	80[a]

Means with the same letters within the same column are not significant different (DNMRT ($P<0.05$)

and DS%, indicating their effectiveness in reducing disease impact. Specifically, (T1) demonstrated the greatest potential as a biocontrol agent (BCA), as highlighted by the lowest disease incidence and severity among the treatments. This suggests that (T1) effectively suppressed the progression of infection caused by *Colletotrichum*, the pathogen responsible for anthracnose in chili plants. The study indicated that its application slowed down the infection progress and also contributed to improved growth of chili plants disease suppression and promising for biological control and plant health promotion.

CONCLUSION

B. stenophylla crude root extracts exhibit significant inhibitory effects against *C. gloesporoides*. *B. stenophylla* crude root extracts effective as inhibitors of mycelial growth of *Colletotrichum*. Treatment with *B. stenophylla* alone delays the growth of *Colletotrichum* fungus. In vitro assays showed a gradual increase in Percentage Inhibition of Radial Growth (PIRG) values until day 8, indicating sustained inhibition of *Colletotrichum* growth over time. *B. stenophylla* demonstrated strong inhibition against *Colletotrichum* in the Dual Culture Test. This indicates its potential as a potent antagonist against *Colletotrichum* species. This dual activity suggests robust potential for biocontrol agents against fungal pathogens. These findings highlight the biocontrol potential of both *B. stenophylla* crude root extracts inoculants in managing fungal diseases in agricultural settings. The extraction ability to inhibit pathogen growth underscores their promising role in integrated pest management strategies. Additionally, the study suggested that *B. stenopyhlla* acted as an effective elicitor, triggering the production of inducible compounds associated with induced resistance in chili plants. This mechanism further supports its role as a biocontrol agent by enhancing the plant's natural defense mechanisms against pathogens. In summary, *B. stenopyhlla* demonstrated significant potential as a biocontrol agent against *Colletotrichum* infection in chili plants. Their effectiveness in disease suppression and growth enhancement underscores their utility in sustainable agriculture practices.

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